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Updated distribution of an alien frog species, *Lithobates catesbeianus* (Shaw, 1802), in Ecuador: new records of Bullfrog in the semideciduous lowland forest of western Ecuador

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Abstract. We report the occurrence of *Lithobates catesbeianus* (Shaw, 1802), Bullfrog, in a protected semideciduous forest in the city of Guayaquil, Guayas, Ecuador and in a nearby wildlife management center. Bosqueira Protected Forest is an isolated spot of diversity surrounded by urbanization, quarries, and highways. We found a juvenile Bullfrog in a water canal on the edge of the protected forest, near a private housing complex, and several tadpoles in an artificial pond in a wildlife management center located near the reserve. This is the first sighting of the invasive *L. catesbeianus* in a native ecosystem in Guayaquil. We also report the this species from, three other provinces of Ecuador, based on observations in iNaturalist. These records reinforce the ability of *L. catesbeianus* to adapt easily to varied environmental conditions and to disturbed areas. The invasion of *L. catesbeianus* towards semideciduous forest represents a new threat to biodiversity, particularly the endemic fauna, due to predation or competition. In future studies, it will be important to fully evaluate the Bullfrog diet and population status to estimate its impact on remnant forests of Guayaquil.

Keywords. Invasive species, Guayas, protected forest, citizen science

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Introduction

Lithobathes catesbeianus (Shaw, 1802), or Bullfrog, is native to North America, specifically to southern Canada, eastern and southern USA, and northern Mexico. Due to its remarkable adaptability and ability to colonize new environments, it has become widely invasive in Europe, Asia, and South America, including Argentina, Belgium, Brazil, China, Colombia, Cuba, the Dominican Republic, Ecuador, France, Germany, Greece, Indonesia, Italy, Jamaica, Japan, Malaysia, the Netherlands, Peru, the Philippines, Puerto Rico, Singapore, Spain, Taiwan, Thailand, the United Kingdom, Uruguay, and Venezuela (Ficetola et al. 2007; Barbosa et al. 2017; Johovic et al. 2020). It has been intentionally introduced into many countries across the globe, primarily for aquaculture (Jennings and Hayes 1985; Siemens 2017). However, once released into the environment, this amphibian species can establish populations that are notoriously difficult to eradicate, leading

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to well-documented negative impacts on native biodiversity due to competition, predation, habitat displacement, and disease or pathogen transmission (Laufer et al. 2008; Iñiguez and Morejón 2012; Valarezo Aguilar and Herrera Vallaradares 2015; Adams and Pearl 2018; Cruz-Cordovez et al. 2020).

The problem with this species lies mainly in its voracious diet, preying on native species and competing for food resources (Zeiner et al. 1990; Young et al. 2004). However, it is also a transmitter of diseases such as chytridiomycosis, which has caused declines and local extinctions of native amphibians (Daszak et al. 2004). Consequently, *L. catesbeianus* is widely considered to be one of the most harmful invasive vertebrates, and the International Union for the Conservation of Nature ranks it as one of the most invasive species worldwide (Lowe et al. 2004). Its introduction to Ecuador was first reported in the 1980s, when adults and tadpoles were transported for the establishment of legal farms for commercial purposes in Guayas, Los Rios, Napo, Pastaza, Morona Santiago, and Zamora Chinchipe provinces (Velasco 2001; Villacis and Zurita 2002; Gallardo 2004; Iñiguez and Morejón 2012; Valarezo-Aguilar et al. 2016). Although the first records of introduced Bullfrogs are mostly associated with disturbed areas, its spread toward natural areas is expected (Cruz-Cordovez et al. 2020).

It has been hypothesized that widespread of *L. catesbeianus* took place because individuals escaped from captivity pools. Some were intentionally transported to other provinces to increase production, while others were released due to the decrease of commercialization (Cisneros-Heredia 2004; Iñiguez and Morejón 2012; Cobos et al. 2015), triggering its dispersal toward natural ecosystems. There are formal reports of feral populations of *L. catesbeianus* in native forests of Ecuador, particularly in the coastal regions in the provinces of Guayas (Cruz-Cordovez et al. 2020), Manabí (Cisneros-Heredia 2004), and Loja (Cobos et al. 2015) and in the Amazonian region in Napo (Cisneros-Heredia 2004) and Zamora Chinchipe (Valarezo-Aguilar et al. 2016). Here, we report the occurrence a feral population of *L. catesbeianus* in a tropical semideciduous protected forest and a wildlife management center (Pantanal Zoo) in Guayaquil, Guayas, Ecuador, based on our fieldwork, and also in Los Rios, Azuay, and Pichincha provinces based on citizen reports from iNaturalist. Furthermore, we emphasize the importance of incorporating citizen sightings into assessing the distribution of invasive species.

Study Area

Bosqueira Protected Forest (13 km^2) is an isolated forest fragment located in the Lowland Semideciduous Forest of Jama-Zapotillo that hosts 13 mammal species, 64 bird species, six amphibian species, and 12 reptile species (GAD Municipal de Guayaquil 2021). Bosqueira Protected Forest, located in Pascuales parish, within the city of Guayaquil, province of Guayas, Ecuador, faces persistent threats from anthropogenic activities, particularly urbanization (GAD Municipal de Guayaquil 2021). It is surrounded by urban developments, quarries, and highways. The forest was declared a protected area in February 2010 (Ministerio del Ambiente de Ecuador 2013; Paz-Ramírez et al. 2018). The floristic composition of the forest includes 74 tree species, 37 shrub species, 69 liana and climber species, 25 herb species, 15 epiphyte and tree parasitic plant species. Approximately 20% of this flora is endemic (Páez-Chávez 2016; Fonseca Arana 2022).

Pantanal Zoo is a wildlife management center (3 km^2) located at km 23 on the road to Daule and is 12 km away from the Bosqueira Protected Forest. The facility showcases a diverse range of both endemic and exotic animal species.

Methods

We conducted surveys in April and July 2022 in Bosqueira Protected Forest and Pantanal Zoo. We surveyed both localities during the day (1300–1800h) and night (1800–2300h) along trails in the protected forest and in the zoo, conducting free and unrestricted searches (Rödel and Ernst 2004). Direct observations were made around water bodies to locate adults and tadpoles of *Lithobathes catesbeianus*. Tadpoles were collected using fishing nets, and adults were collected by hand.

Specimens were euthanized using a 2% lidocaine solution, fixed in 90% alcohol, and preserved in 70% ethanol (Ayala-Varela and Paucar 2021). Muscle and liver tissue were collected and preserved in 90% ethanol. Voucher specimens were collected under permit No. MAAE-DBI-DBI-CM-2022-0222 issued by the Ecuadorian Ministry of the Environment, Water and Ecological Transition and are deposited at the Museo de Zoología, Pontificia Universidad Católica del Ecuador, Quito (QCAZ).

Stomach and intestine contents of one individual were extracted, analyzed under a Mshot MS60 stereomicroscope, and stored in 90% alcohol. Items were identified to the lowest taxonomic level possible following Triplehorn and Johnson (2005).

A distribution map was created using records reported in the literature for *L. catesbeianus* in Ecuador, research-grade observations obtained from iNaturalist (compiled in February 2023), and our new localities reported in this study. To ensure accuracy, iNaturalist observations were considered only when identification was confirmed. Records were summarized and grouped based on their geographical proximity, considering 0.5 km radius (Appendix Table A1). This approach prevented points from overlapping, and erroneous record inclusion. This approach helped us ensure a reliable dataset, as citizens often report observations that are near each other or may provide mistaken taxonomic identity, potentially leading to duplicate or erroneous records. We plotted the records considering land

cover (agriculture, anthropogenic activities and native vegetation) provided by the Ministerio de Ambiente, Agua y Transicion Ecologica (2021) using QGIS v. 3.22.16 (2023).

Results

Materials examined. ECUADOR – **Guayas** • Guayaquil Canton, Pantanal Zoo; 02°00′10″S, 079°57′52″W; alt. 24 m; 24.IV.2022; S. Cuadrado leg.; found on an artificial large pond; 4 (tadpoles), QCAZ 77971–77974 (Fig. 1A) • Guayaquil Canton, Bosqueira Protected Forest; 02°00′42″S, 079°58′35″W; alt 68 m; 24.IV.2022; S. Cuadrado leg.; found on an artificial water canal; 1 juvenile (sex indet.), QCAZ 77847 (Fig. 1B).

Diet. Four coleopterans (Elateridae) were found in the stomach of QCAZ 77847 (Fig. 1C).

Identification. This species is characterized by its large size, with a snout–vent length of 10–20 cm and a weight of 60–900 g. Its head is broad and flat, with a fold on each side of the eye bordering the tympanum. While this species displays multiple coloration patterns, the dorsum of adults is typically olive-green to greenish brown with brown or dark green spots; the ventrum is cream-colored with yellow or gray spots. Juveniles are usually olive-green with black dots covering the dorsum, similar to the juvenile we collected (Fig. 1A). The back can either be smooth or grainy, and the hind legs have five toes joined by a broad interdigital membrane, except for the fourth toe. The forelimbs have four completely free toes, and both the forelimbs and hindlimbs end in rounded tips. Sexual dimorphism is evident, as the tympanum is larger in diameter than the eye in males but equal in size to the eye in females (Bury and Whelan 1986; SEDRU 2005; CIDEM 2010; AmphibiaWeb 2023).

The tadpole of this species is large, measuring 5–17 cm. Its body is oval and darkly colored; there is a green or olive dorsum with a maize-colored venter, mottling over the body, and many small dark spots and fine yellow dots (Bury and Whelan 1986). The head is prominent with a small ventral depression, which gives rise to the mouth. The tail is prominent and flattened. There is also a small adhesive disc to adhere to any surface. The tadpoles that we collected were 7.1–8.5 mm long and had yellow dots (Fig. 1B).

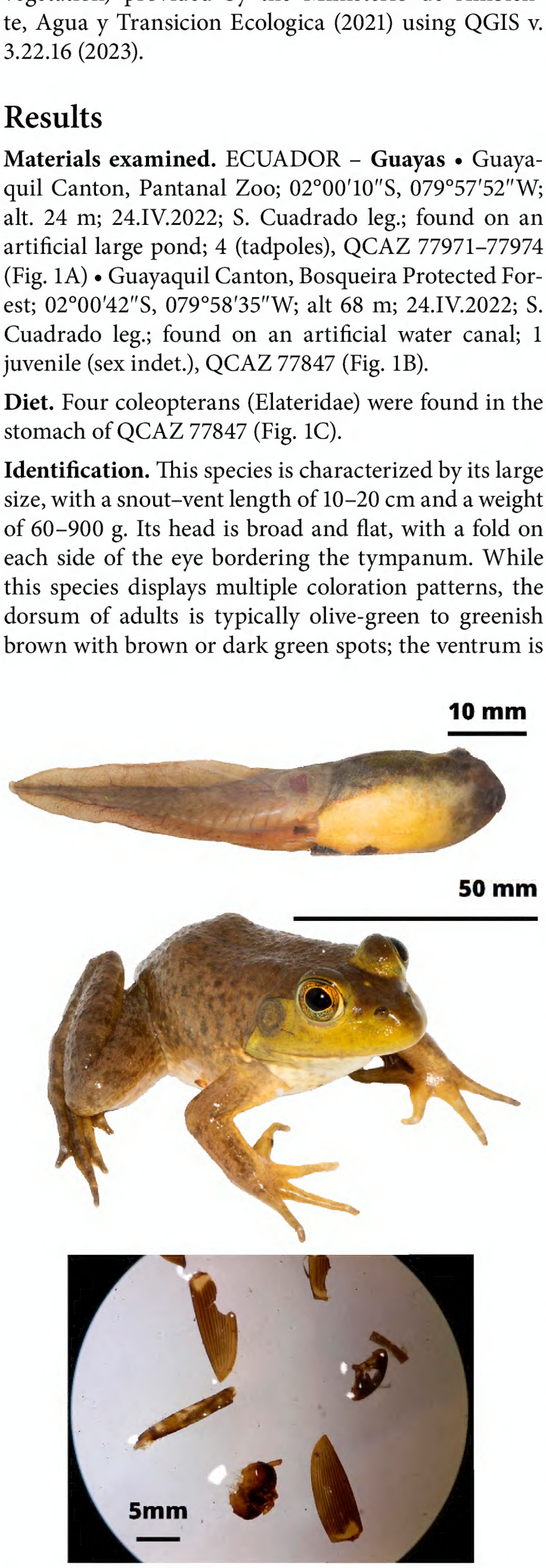

Figure 1. *Lithobates catesbeianus*. **A.** A tadpole found in Pantanal Zoo. **B.** A juvenile frog from Bosqueira Protected Forest. **C.** Food items (Coleoptera, Elateridae) found in the stomach of one individual (QCAZ 77847).

Distribution. We compiled 112 observations of *Lithobathes catesbeianus* from iNaturalist and grouped these into 28 locations based on their proximity to one another, and we report two new occurrences of *L. catesbeianus* in Bosqueira Protected Forest and Pantanal Zoo, based on our collections (Fig. 2). Pantanal Zoo, a site modified for wildlife management, features a large pond that has been colonized by this species.

Observations in iNaturalist indicate that *L. catesbeianus* has been observed in additional locations in Guayas, including urban areas close to forest edges and another protected reserve (Area de Recreacion Parque Lago), as well as in Loja and Zamora Chinchipe provinces where this species has been previously reported in the literature. Combined, the new sightings allow us to extend the geographic range of *L. catesbeianus* has to Los Rios, Azuay, and Pichincha provinces (Fig. 2, Appendix Table A1). In all provinces, occurrence of this species overlaps with areas of high disturbance, except for Isla Santay.

Discussion

The new records of *Lithobates catesbeianus* from Guayas presented here are located 32 km away from the nearest previously confirmed report of a feral population in the native forests of Isla Santay (Duran district, Guayas province) (Cruz-Cordovez et al. 2020). However, based on observations in iNaturalist, current distribution of this species includes three more provinces in the coastal and Andean region, both in disturbed areas and native ecosystems. Thus, feral populations of *L. catesbeianus* are widespread in Ecuador, and ongoing invasions by this species are possible. Nonetheless, a thorough study on the status of populations of this species is recommended, particularly in areas such as Isla Santay, Area de Recreacion Parque Lago, and Bosqueira

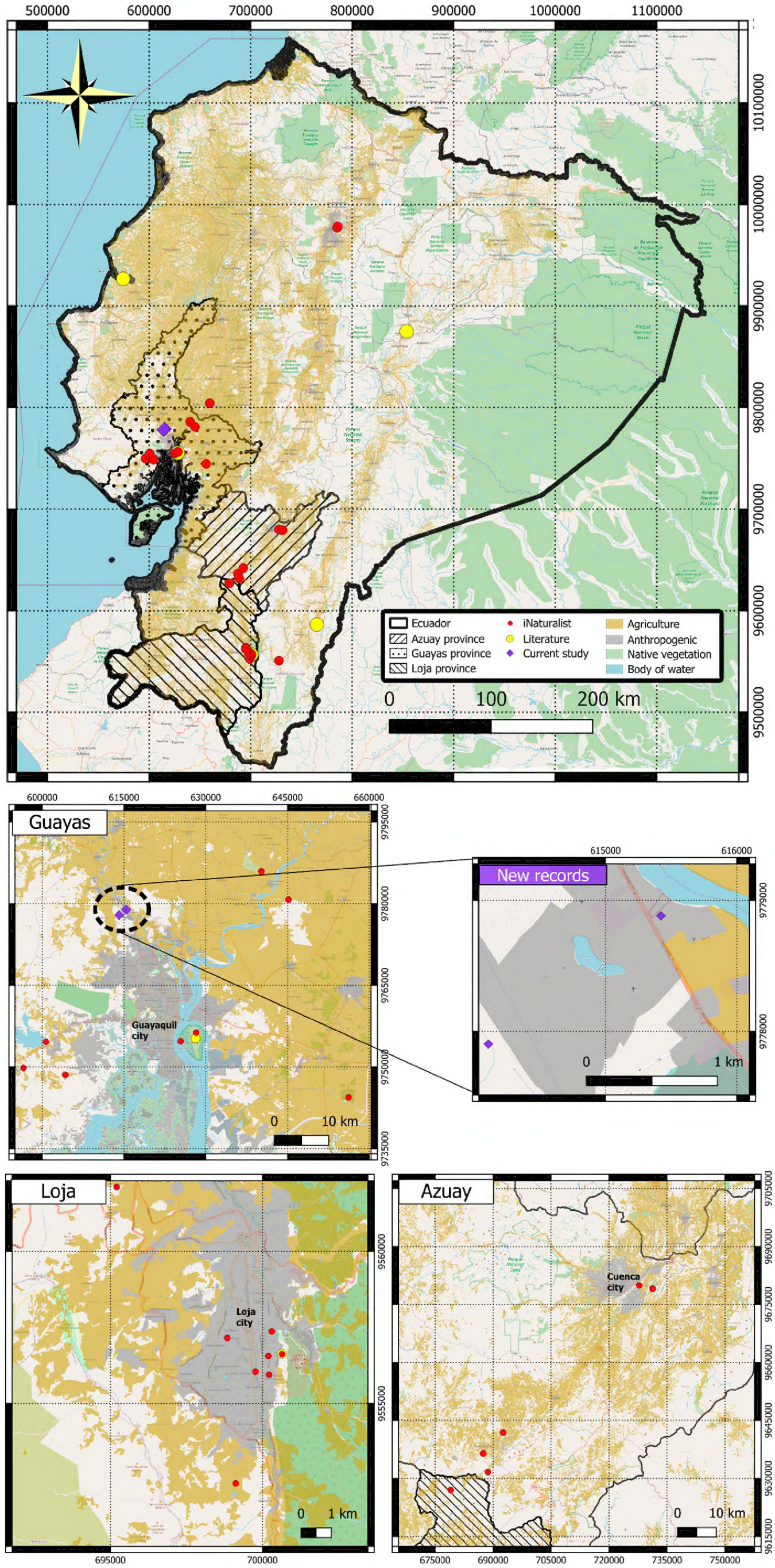

Figure 2. Geographic distribution of *Lithobates catesbeianus* in Ecuador. Red dots = iNaturalist observations, yellow dots = historical reports, and green dots = new records (green). Land disturbance where the bullfrog has been reported is included (Ministerio del Ambiente 2012).

Protected Forest where native species may be affected by its introduction.

The ability of *L. catesbeianus* to adapt to varied environments and its potential for out-competing with native frog species for food and habitat use has been well-documented (Bissanttini et al. 2019). Specifically, the dependence of *L. catesbeianus* on permanent water resources (Bury and Whelan 1986) makes its presence of artificial water bodies associated with urban infrastructure in Bosqueira Protected Forest highly conducive to its reproduction and occurrence. Consequently, the interconnectedness between this protected forest and the population from Pantanal Zoo poses an increased risk of further spread into the native forest. *Lithobates catesbeianus* has been reported as a generalist predator, targeting both native frogs (Oda et al. 2019; Sarashina and Yoshida 2021) and coleopterans (Laufer et al. 2021; Cruz et al. 2021). However, in our examined sample, only coleopterans were found. As a result, drawing conclusions about competition or predation based on this one sample is not possible. To understand feeding behavior of *L. catesbeianus* in Bosqueira, a thorough evaluation of its population is required.

We note the importance of citizen engagement and specifically iNaturalist, as such data can include areas that researchers are unable to personally survey (Fourcade 2016; Di Cecco et al. 2021), such as Azuay and Los Rios provinces in our study. Engaging citizens not only increases the coverage of data collection but also proves to be cost effective. However, there is the potential for biased data arising from individuals' affinity towards specific taxonomic groups (Shumskaya et al. 2023). By involving citizens, we can select and visit sites and allow for timely responses for population control of invasive species and the protection of native wildlife.

The invasion of *L. catesbeianus* poses a new and significant threat to the semideciduous forest. As previously reported, this species preys upon a wide variety of native species and additionally acts as a resilient carrier of the fungus *Batrachochytrium dendrobatidis*, which is responsible for causing chytridiomycosis—a fatal disease among native amphibians (Ribeiro et al. 2019). These threats to the native fauna makes the invasion of *L. catesbeianus* particularly concerning, as it jeopardizes the ecological integrity of the semideciduous forest. Compared to endemic or native species, *L. catesbeianus* possesses a distinct advantage to colonized disturbed ecosystems. It reproduces in artificial aquatic habitats, withstands drought conditions, and has a generalist diet. In contrast, native species in the deciduous forest must endure long periods of dry seasons, require specific conditions to reproduce, and have a restricted ecological range and dietary requirements. We recommend conducting further research in Bosqueria Protected Forest to examine the genetic, ecological, and behavioral variation patterns that support the dispersal of *L. catesbeianus* into native forest in Guayaquil and assess its impact on the biodiversity of the forst of Bosqueira.

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Author Contributions

Conceptualization: AN. Data curation: AN, SC. Formal analysis: AN, SC, MB, KV. Funding acquisition: AN, NM. Project administration: AN. Supervision: AN. Visualization: MB. Writing – original draft: AN. Writing – review and editing: MB, NM, SC, KV.

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Appendix

Table A1. List of *Lithobates catesbeianus* observations in iNaturalist.

Source	ID	Province	Canton	Parish	Locality	Latitude	Longitude
iNaturalist	15684043	Azuay	Cuenca	Paccha	Amaru Zoo	−02.897	−078.953
	60288244	Azuay	Cuenca	Paccha	Paccha	−02.905	−078.922
	10254778	Azuay	Santa Isabel	Santa Isabel (Chaguarurco)	Hacienda. Los Faiques	−03.291	−079.315
	133571006	Azuay	Santa Isabel	Abdon Calderon (La Union)	Lagoon Crea- Abdon Calderon (yunguilla)	−03.242	−079.269
	41268570	Azuay	Santa Isabel	Santa Isabel (Chaguarurco)	Sulupali Grande	−03.335	−079.304
	76037821	Guayas	Durán	Eloy Alfaro (Durán)	Isla Santay	−02.222	−079.875
	27072421	Guayas	Durán	Eloy Alfaro (Durán)	Isla Santay	−02.207	−079.849
	68734034	Guayas	El Triunfo	El Triunfo	Hacienda Bamboo 2	−02.315	−079.598
	54125902	Guayas	Guayaquil	Tarqui	Urbanization Lomas del bosque	−02.267	−080.134
	62586407	Guayas	Guayaquil	Tarqui	Parque Lago	−02.223	−080.097
	27970963	Guayas	Samborondon	Tarifa	Samborondon	−01.940	−079.742
	148431546	Guayas	Samborondon	Samborondon	Agricultural area	−01.987	−079.697
	106572722	Guayas	Guayaquil	Pascuales	Pantanal Zoo	−02.004	−079.964
	119153579	Guayas	Guayaquil	Tarqui	Finca Botanica Aromatica	−02.278	−080.065
	145703771	Loja	Loja	Carigan	Parque de Carigán	−03.963	−079.244
	115555341	Loja	Loja	San Sebastián	Barrio Yahuarcuna	−04.019	−079.199
	115555342	Loja	Loja	San Sebastián	Barrio La Pradera	−04.013	−079.199
	21845798	Loja	Loja	Carigan	Lago Valle Hermoso	−03.953	−079.243
	114457612	Loja	Loja	San Sebastián	La Cruz (Lake)	−04.051	−079.209
	145451408	Loja	Loja	San Sebastián	Parque Pucará	−04.013	−079.195
	84062916	Loja	Loja	San Sebastián	Parque Lineal La Tebaida	−04.018	−079.203
	115040931	Loja	Loja	San Sebastián	Parque Pucará	−04.006	−079.198
	86170100	Loja	Loja	Sucre	Barrio Epoca	−04.008	−079.211
	30183146	Loja	Saraguro	San Sebastián de Yúluc	Parque Eólico Huascachaca	−03.377	−079.391
	71449539	Los Ríos	Babahoyo	Pimocha	Agricultural area	−01.774	−079.567
	52268165	Pichincha	Quito	Cumbayá	Alemán de Quito College	−00.211	−078.441
	105615910	Pichincha	Quito	Cumbayá	San Francisco de Quito University	−00.197	−078.436
	20017134	Zamora Chinchipe	Zamora	Zamora	Malecón de Zamora	−04.066	−078.950
Cisneros-Heredia 2004		Napo	Carlos Julio Arosemena Tola	Carlos Julio Arosemena Tola	Puyo-Tena Road	−01.134	−077.825
		Manabí	Sucre	Bahía de Caraquez	Shrimp farm	−00.667	−080.333
Cruz-Cordovez et al. 2020		Guayas	Durán	Eloy Alfaro (Durán)	Isla Santay	−02.217	−079.850
Cobos et al. 2015		Loja	Loja	Loja	Pucará Park	−04.013	−079.195
Valarezo-Aguilar et al. 2016		Zamora Chinchipe	Yantzaza	Los Encuentros	Estación Experimental El Padmi	−03.743	−03.743
Current survey	QCAZ 77847	Guayas	Guayaquil	Pascuales	Bosqueira Protected Forest	−02.012	−079.976
	QCAZ 77971	Guayas	Guayaquil	Pascuales	Pantanal Zoo	−02.004	−079.964